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NEW DATA ON *HENNEGUYA PELLIS* (MYXOZOA: MYXOBOLIDAE), A PARASITE OF BLUE CATFISH *ICTALURUS FURCATUS*

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ABSTRACT: The original description of *Henneguya pellis*, a myxozoan parasitizing blue catfish *Ictalurus furcatus*, is supplemented with new data on histopathology, spore morphology, and 18S small subunit (SSU) ribosomal DNA (rDNA) sequence. Plasmodia presented as both internal and external, raised, cyst-like lesions on the body wall of the peritoneal cavity and on the skin. The cysts contained numerous elongate, lanceolate myxospores, flattened parallel to the suture line. The spore body was 14.8 ± 1.1 μm (range 13.0–17.1) long and 4.8 ± 0.8 μm (range 4.0–7.4) wide in frontal view. The caudal appendages were 77.7 ± 8.8 (range 57.4–96.4) in length. There were 2 pyriform polar capsules, unequal in length, with the longer capsule measuring 7.2 ± 0.6 μm (range 6.2–8.4) in length and the shorter capsule measuring 6.5 ± 0.5 μm (range 5.5–8.0). The polar capsules were not significantly different in width, measuring 1.7 ± 0.2 μm (range 1.4–1.9). There were 8 turns in the polar filament coil. The total length of the spore was 92.5 ± 9.2 μm (range 73.3–113.5). Spore morphology and site of development are similar to that of *Henneguya sutherlandi* from channel catfish; however, 18S rDNA sequence data support previous findings that identify *H. pellis* and *H. sutherlandi* as 2 distinct species.

Henneguya is one of the most diverse genera of the Myxosporea, consisting of at least 204 described species known to infect both fresh and marine fishes (Eiras, 2002; Lom and Dykova, 2006). The commercial catfish industry provides an optimal environment for the propagation of myxozoan parasites, with its closed earthen ponds and multi-batch culture. As such, several species of *Henneguya* have been identified from commercially raised catfish (Kudo, 1920, 1929; Meglitsch, 1937; Guilford, 1965; Minchew, 1977; Current, 1979; Lin et al., 1999; Pote et al., 2000; Griffin et al., 2008). Of the species of *Henneguya* known to infect ictalurids of the United States, 5 have been identified by 18S rDNA sequence; *Henneguya ictaluri* (Pote et al., 2000), *Henneguya sutherlandi* (Griffin et al., 2008), *Henneguya adiposa* (Minchew, 1977; Griffin et al., 2009), *Henneguya exilis* (Kudo, 1929; Lin et al., 1999), and *Henneguya gurleyi* (Kudo, 1920; Iwanowicz et al., 2008). All species were isolated from channel catfish *Ictalurus punctatus* except *H. gurleyi*, which was described from wild brown bullhead *Ameiurus nebulosus*.

Of the species of *Henneguya* known to infect ictalurid fishes, only *Henneguya pellis* (Minchew, 1977) has been reported from the blue catfish *Ictalurus furcatus*. Host specificity appears to be variable within the myxozoans (Molnar et al., 2002), suggesting that *H. sutherlandi*, isolated from the closely related channel catfish (Griffin et al., 2008), and *H. pellis* from the blue catfish (Minchew, 1977) are actually the same organism infecting different hosts. The present paper supplements the original description of *H. pellis* with new data on histopathology, morphology, and 18S rDNA sequence, supporting the original claims by Minchew (1977) and Griffin et al. (2008) that *H. pellis* and *H. sutherlandi* are 2 distinct species, separate from the other *Henneguya* species known to infect ictalurid fishes (Minchew, 1977; Lin et al. 1999; Pote et al. 2000; Iwanowicz et al., 2008; Griffin et al., 2009).

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MATERIALS AND METHODS

In August 2008, three brood size (2–3 kg) blue catfish from a commercial operation were submitted to the Aquatic Diagnostic Lab of the Thad Cochran National Warmwater Aquaculture Center for health assessments. The fish were found to have several ovoid, slightly irregular dermal and peritoneal wall pseudocysts, measuring 3–5 mm in diameter. These pseudocysts contained numerous lanceolate myxospores morphologically consistent with *Henneguya pellis* infection. Morphological and genetic analysis was subsequently performed on the collected spores, and infected tissues were processed for histological examination.

Individual internal and external pseudocysts were excised from each fish by sharp dissection, placed on a microscope slide with a drop of physiological saline, 0.85% (w/v) of NaCl, and mechanically ruptured. The myxospores were further diluted with saline, cover slipped, and examined using an Olympus BX-50 microscope. Representative images (n = 25) of spores from all 3 fish were captured using a Spot insight QE digital camera and analyzed using Spot Basic 3.1 image analysis software (Diagnostic Instruments, Sterling Heights, Michigan).

Representative samples of infected skin and peritoneal wall were removed by sharp dissection and placed in 10% neutral buffered formalin for a minimum of 24 hr. Tissue samples were trimmed, processed, embedded in paraffin, and sectioned at 6 μm. Prepared slides were stained with hematoxylin and eosin (H&E) and Giemsa stains (Luna, 1968).

Spores from microscopic analysis, as well as intact plasmodia excised from the affected skin and peritoneal wall, were placed in 600 μl of Puregene® cell lysis solution (Gentra Inc., Minneapolis, Minnesota) and incubated for 10 min at 95 C. Following lysis, 3 μl (20 mg/ml) of proteinase K was added to the homogenate prior to overnight incubation at 55 C. The remainder of the isolation was carried out according to the manufacturer's suggested protocol. The purified genomic DNA was then resuspended in 30 μl of Puregene® DNA hydration solution.

The 18S rDNA gene was first amplified with the universal eukaryotic primers ERIB1 and ERIB10 (Barta et al., 1997; Fiala, 2006). Nested PCR reactions were conducted using primer sets H2/H9 (Hanson et al., 2001) and MyxospecF-MyxospecR (Fiala, 2006), as well as combinations of the aforementioned with the generic myxozoan primers Genmyxo3, Genmyxo4, and Genmyxo5 as described by Griffin et al. (2008) (Table I). The initial 25-μl PCR reaction mixture contained 2.5 μl of TaKaRa Taq® Hot Start version 10X PCR buffer (TaKaRa Bio Inc., Otsu, Shiga, Japan), 5 mmol dNTPs, 10 pmol of each primer (ERIB1 and ERIB10), 0.625 U of TaKaRa Taq® Hot Start *Taq* polymerase, 13.75 μl of nuclease free H_2O, and 2 μl of DNA template. The reaction mixture was cycled on a MJ Research PTC-200 thermal cycler (GMI Inc., Ramsey, Minnesota), with an initial denaturation step of 95 C for 10 min followed by 30 cycles of 95 C for 1 min, 48 C for 1 min, 72 C for 2 min, and a final extension step of 72 C for 10 min. One microliter of PCR product from the initial reaction was used in nested PCR with the following primer combinations; ERIB1/Genmyxo5, H2/H9, MyxospecF/MyxospecR, Genmyxo3/H2, and Genmyxo4/H2 producing PCR products of approximately 500, 700, 800, 1,000, and 1,000 base pairs, respectively. All reaction components remained the same, except 14.75 μl nuclease free H_2O was used to bring the reaction volume to 25 μl and the

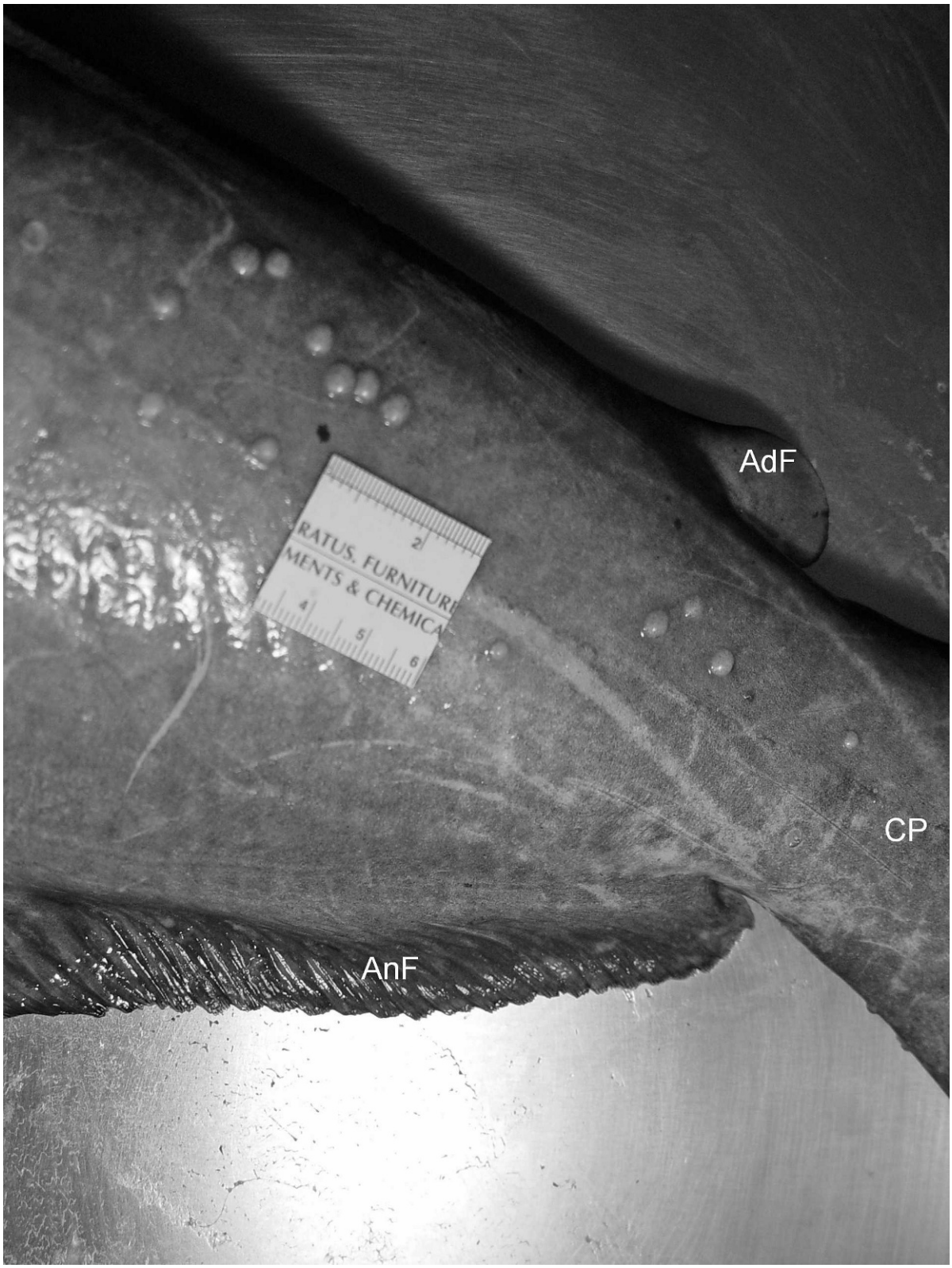

FIGURE 1. Gross presentation of *Henneguya pellis* on the skin of blue catfish. AdF = adipose fin; AnF = anal fin; CP = caudal peduncle.

TABLE I. Primer sequences.

Primer	Sequence (5′–3′)	Direction	Sequence location (bp)*	Reference
H9	TTACCTGGTCCGGACATCAA	Forward	1,331–1,350	Hanson et al., 2001
H2	CGACTTTTACTTCCTCGAAATTGC	Reverse	2,021–2,024	Hanson et al., 2001
MyxospecF	TTCTGCCCTATCAACTWGTTG	Forward	301–322	Fiala, 2006
MyxospecR	GGTTTCNCDGRGGGMCCAAC	Reverse	1,214–1,195	Fiala, 2006
Genmyxo3	TGATTAAGAGGAGCGGTTGG	Forward	982–1,001	Griffin et al., 2008
Genmyxo4	GGATGTTGGTTCCGTATTGG	Forward	957–976	Griffin et al., 2008
Genmyxo5	TAAGCGCAGCAACTTTGAGA	Reverse	617–598	Griffin et al., 2008
ERIB1	ACCTGGTTGATCCTGCCAG	Forward	2–20	Barta et al., 1997
ERIB10	CCTCCGCAGGTTCACCTACGG	Reverse	2,079–2,059	Barta et al., 1997

* Sequence location based on the 18S SSU rDNA sequence of *Henneguya exilis* (AF021881).

annealing temperature was 52 C instead of 48 C. The PCR product was run on a 1.2% agarose gel and stained to confirm the presence of DNA product.

The PCR products were purified using a Montage PCR centrifugal filter device (Millipore, Billareca, Maryland), resuspended in 20 µl Puregene® DNA hydration solution, and quantified using a NanoDrop® spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware). Each product was then sequenced directly 3 times in each direction by dideoxy chain termination sequencing (Sanger et al., 1977) with an ABI prism dye termination cycle sequencing kit (Applied Biosystems, Foster City, California) using ~40 ng of template per reaction. The products were purified using Centri-Sep spin columns (Princeton Separations, Adelphia, New Jersey) and analyzed at the USDA-ARS Mid South Area Genomics Laboratory, Stoneville, Mississippi. The sequence fragments were assembled into a single contiguous sequence using the SeqMan utility of the Lasergene v7.0 software package (DNASTAR Inc., Madison, Wisconsin).

The obtained DNA sequence was compared with sequences from the NCBI non-redundant nucleotide (nr/nt) database using the megablast search for highly similar sequences. The phylogenetic relationship of the ictalurid-infecting myxozoans to myxobolids isolated from other families of fish was estimated. All available 18S rDNA sequences from described *Henneguya* species, along with the 15 most similar sequences from described *Myxobolus* species, were downloaded from the NCBI database and aligned using the Clustal W application of the Molecular Evolutionary Genetics Analysis, 4.0 (MEGA4) software package (Tamura et al., 2007). The following sequences were obtained, aligned, and used in the construction of a phylogram: *H. adiposa* (EU492929), *Henneguya akule* (EU016076), *Henneguya cutanea* (AY676460), *Henneguya daoudi* (EU643625), *Henneguya doneci* (EU344899), *Henneguya doori* (HDU37549), *H. exilis* (AF021881), *H. ictaluri* (AF195510), *H. gurleyi* (DQ673465), *Henneguya lateolabracis* (AB183747), *Henneguya lesteri* (AF306794), *Henneguya nuesslini* (AY669810), *Henneguya pagri*

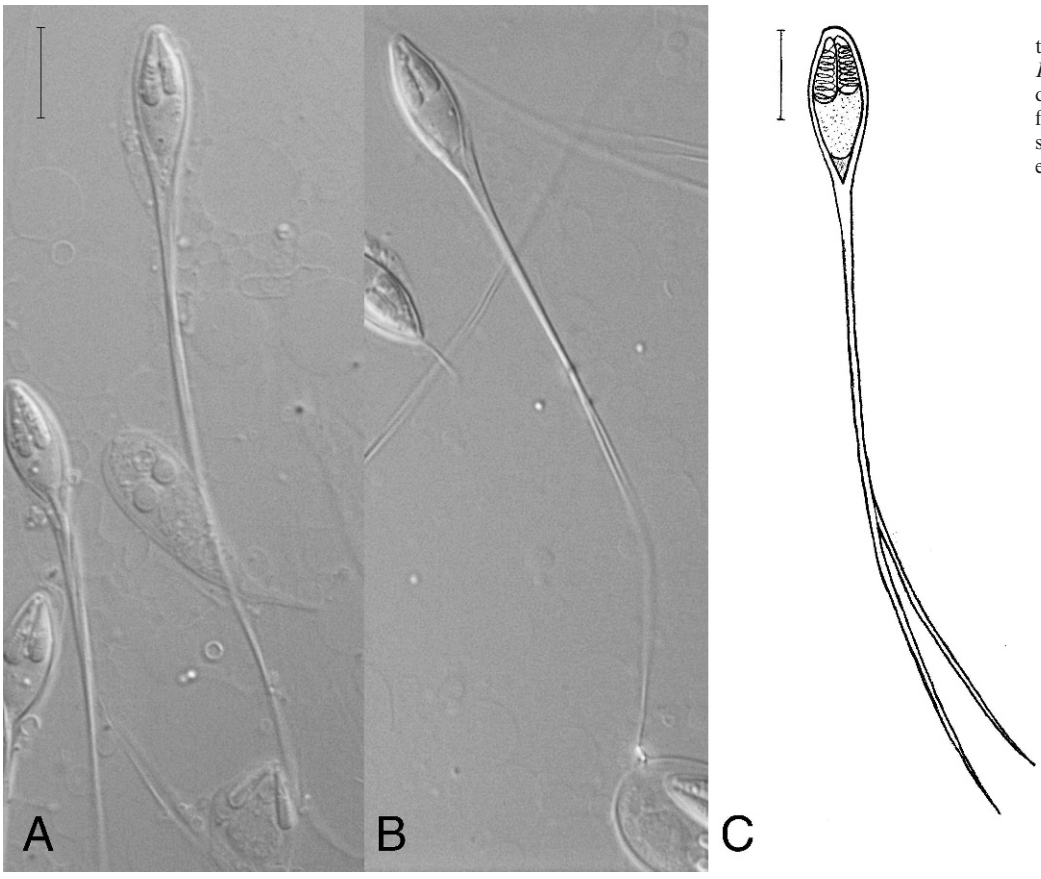

FIGURE 2. Wetmount preparations (**A, B**) and schematic (**C**) of *Henneguya pellis* myxospores demonstrating the coiled polar filaments within the polar capsule, spore capsule, and caudal processes; scale bars ~10 µm.

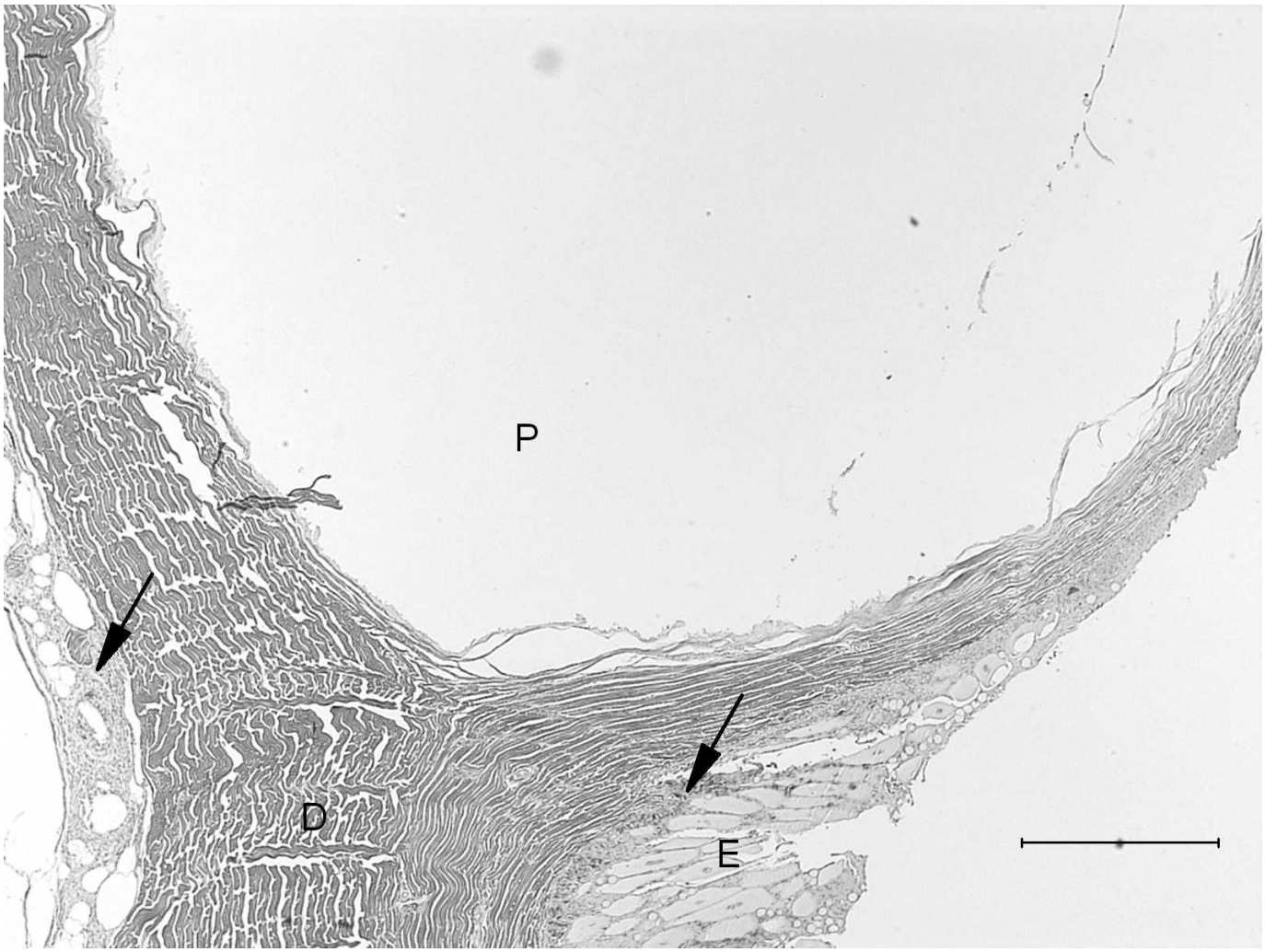

FIGURE 3. Section through a pseudocyst in the superficial dermis where the collagen bundles are separated by the formation of this structure. Small inflammatory infiltrates indicated by arrows (H&E; scale bar ~250 μm; D, dermis; E, epidermis; P, pseudocyst).

(AB183748), *Henneguya rhinogobii* (AB447993), *Henneguya salminicola* (AF031411), *H. sutherlandi* (EF191200), *Henneguya shaharini* (EU643630), *Henneguya weishanensis* (AY165182), *Henneguya zschokkei* (AF378344), *Myxobolus arcticus* (AB353129), *Myxobolus bizerti* (AY129318), *Myxobolus bibullatus* (AF378336), *Myxobolus cerebralis* (EF370481), *Myxobolus cultus* (AB121146), *Myxobolus episquamalis* (AY129312), *Myxobolus exiguus* (AY129317), *Myxobolus fryeri* (EU346372), *Myxobolus ichkeulensis* (AY129315), *Myxobolus kisutchi* (EF431919), *Myxobolus lentisuturalis* (AY278563), *Myxobolus leptobarbari* (EU643623), *Myxobolus neurobius* (AF085180), *Myxobolus osburni* (AF378338), *Myxobolus spinacurvatura* (AF378341). *Tetracapsuloides bryosalmonae* (U70623) served as an outgroup for phylogenetic analysis.

Phylogenetic and molecular evolutionary analyses were conducted on the obtained 18S rDNA sequence using the MEGA4 software package (Tamura et al., 2007). The data were analyzed by maximum parsimony analysis (MP) using a close-neighbor-interchange (CNI) search level 3 with the random addition of 100 trees. Minimum evolution (ME) distance analysis was also performed using a CNI search level 3, with evolutionary distances determined by maximum composite likelihood (Rzhetsky and Nei, 1992; Nei and Kumar, 2000). The initial tree for ME analysis was generated using the neighbor-joining algorithm with pair-wise gap deletion (Saitou and Nei, 1987; Tamura et al., 2004). Clade support for both analyses was assessed by bootstrapping (1,000 replicates for ME; 100 replicates for MP) (Felsenstein, 1985).

Sequence identities (% similarity) to *H. pellis* were determined for sequences of *Henneguya* species obtained from GenBank. Sequences were aligned using the Clustal W utility of the MEGA4 software package (Tamura et al., 2007), and results were based on pair-wise analysis of the nucleotide sequences. The percentage similarities between the *H. pellis* 18S rDNA sequence and that of selected myxozoan species was determined.

RESULTS

All fish examined presented with multiple white, ovoid, slightly irregular pseudocysts within the dermis and the interior of the peritoneal wall, each measuring 3–5 mm in diameter (Fig. 1). Removal and subsequent rupture of the cysts during wet mount preparation revealed numerous lanceolate myxospores with 2 polar capsules and elongate caudate processes, consistent with species of *Henneguya* (Fig. 2).

Wet mount examination revealed myxospores morphologically consistent with those of *H. pellis* (Minchew, 1977), except that spore body length was slightly longer and caudal process length was shorter. The myxospore possessed an elongate, lanceolate spore body, flattened parallel to the suture line: 14.8 ± 1.1 μm (range 13.0–17.1) long and 4.8 ± 0.8 μm (range 4.0–7.4) wide in frontal view. The caudal appendages were split posteriorly and were 77.7 ± 8.8 (range 57.4–96.4) in length. Pyriform polar capsules were

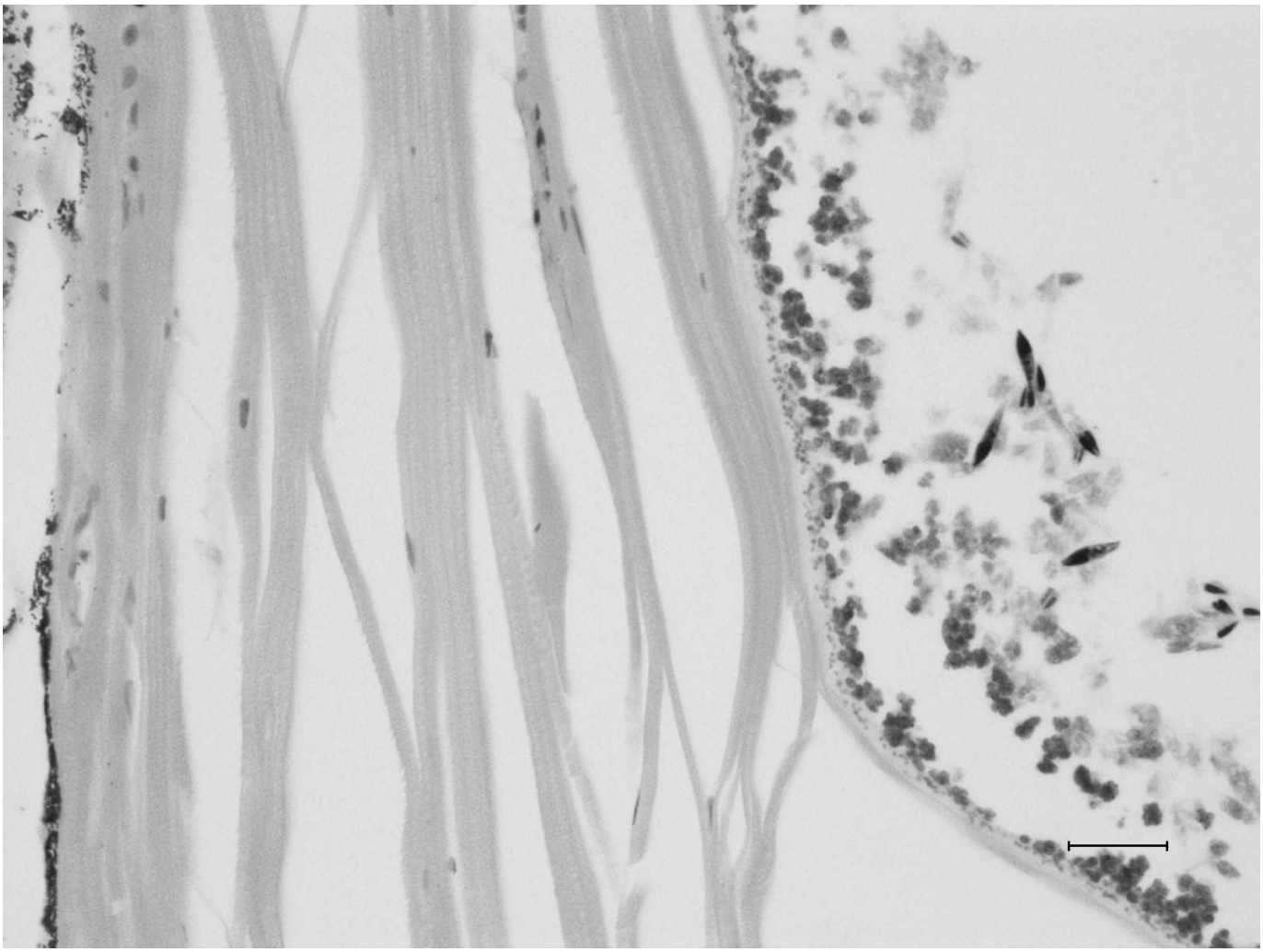

FIGURE 4. Higher magnification of the dermal pseudocyst with the few spores present in the periphery (Giemsa; scale bar ~25 μm).

unequal in length, with the longer capsule measuring 7.2 ± 0.6 μm (range 6.2–8.4) in length and the shorter capsule measuring 6.5 ± 0.5 (range 5.5–8.0). The polar capsules were not significantly different in width, measuring 1.7 ± 0.2 μm (range 1.4–1.9). There were 8 turns in the polar filament coil. The total length of the spore was 92.5 ± 9.2 μm (range 73.3–113.5).

Histologically, the external pseudocysts were raised, vesicular projections caused by developing plasmodia creating discrete cyst-like lesions between collagen bundles in the middle of the dermis (Fig. 3). Portions of these raised lesions exhibited complete or partial loss of the epidermis. Mild mononuclear inflammatory infiltrates were present in the adjacent intact epidermis. Multifocal, small, similar type infiltrates were sometimes observed between the collagen fibers surrounding these expansile lesions. Occasionally, there were similar small inflammatory infiltrates within the subjacent hypodermis. No free spores were readily evident (in the H&E and Giemsa stained sections) in these inflammatory foci. In fact, there were relatively few spores within the pseudocysts (presumed lost during trimming, processing, and sectioning) with the few remaining spores present in the periphery of the lesion (Fig. 4). These spores are described above and are characterized by extremely long caudal processes almost 4 times the length of the spore body.

The pseudocysts from the peritoneal wall were not as well circumscribed on histological examination as those in the superficial dermis (Fig. 5). There was an intense granulomatous inflammatory infiltrate consisting of a mix of inflammatory cells including multinucleated giant cells dissecting between the surrounding tissues. Within this inflammatory milieu were rare, loose spores that were difficult to discern on H&E stained sections but were readily recognizable on Giemsa stained sections (Fig. 6).

The obtained 18S rDNA sequence (2023 bp) did not produce any exact matches during a megablast search for highly similar sequences of the NCBI non-redundant nucleotide (nr/nt) database but was most closely related to the *Henneguya* species known to infect ictalurid fishes. These species formed a distinct phylogenetic branch when compared to similar myxozoan sequences of comparable length obtained from the NCBI database. Iwanowicz et al. (2008) identified 3 distinct variable regions within the 18S rDNA locus diagnostic for species discrimination within the Myxobolidae. The diagnostic variable regions (DVR) used in this analysis correspond to nucleotides 150–300 (DVR 1), 600–1,050 (DVR 2), and 1,350–1,800 (DVR 3) on the *H. exilis* 18S rDNA sequence (AF021881), respectively. Multiple bases differed in these 3 regions between *H. pellis* and the 5 ictalurid-infecting

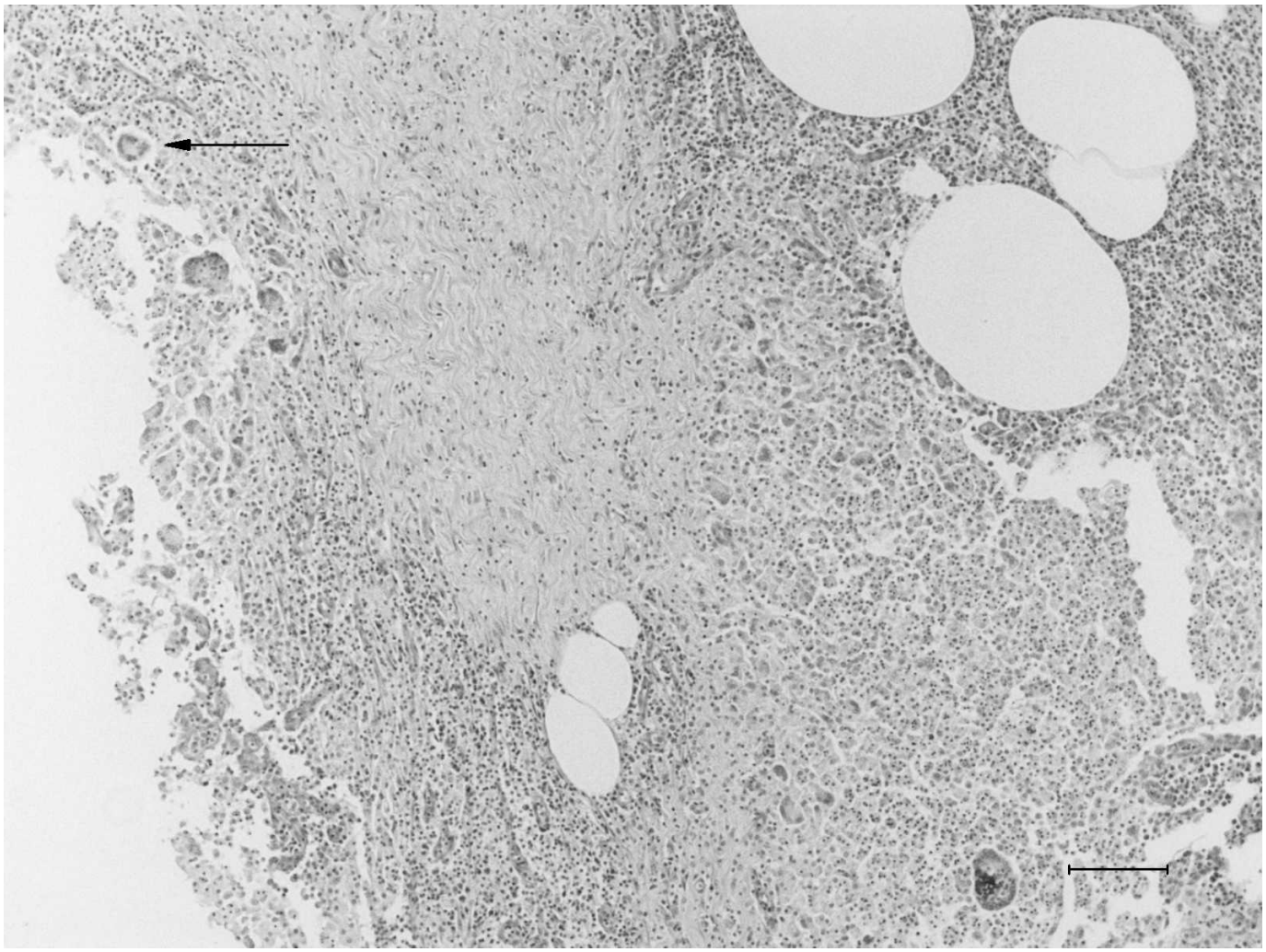

FIGURE 5. Section through a peritoneal pseudocyst. Note the more intensive inflammatory component compared to the dermal pseudocyst. The arrow indicates a multinucleated giant cell (H&E; scale bar ~100 μm).

Henneguya species (Fig. 7) for which sequence data are available. Both maximum parsimony (Fig. 8) and minimum evolution (Fig. 9) analysis were in agreement of the phylogenetic placement of *H. pellis* within the clade of ictalurid-infecting *Henneguya* species as a sister species to *H. sutherlandi* (100% bootstrap support by both MP and ME analysis). Pair-wise comparison of 18S rDNA sequence revealed *H. pellis* to be most similar to *H. sutherlandi* (97.1% similarity over 1,993 base pairs). Of the nearly 3% variation between the 18S rDNA sequence of *H. pellis* and *H. sutherlandi*, most of the variability was localized over the 3 aforementioned variable regions, with 4% (7/150 bases), 3% (10/300 bases), and 9% (27/300 bases) differences at regions 1, 2, and 3, respectively. Although *H. pellis* exhibits significant sequence similarities to members of the genus isolated from ictalurids (>90%), it does not share the same level of similarity with other members of the genus isolated from non-ictalurid fish (Table II).

DISCUSSION

Spores of *H. pellis* are similar (overlap in measured ranges of 4 or more of 6 diagnostic features) to those of *Henneguya diversis*, *H. exilis*, *H. sutherlandi*, *H. gurleyi*, *Henneguya longicauda*, *Henneguya postexilis*, *H. pellis*, and *Henneguya limatula*, all of which parasitize ictalurids in North America. Comparisons of host record, spore morphology, and plasmodial localization of *H. pellis* and other ictalurid-infecting *Henneguya* species are presented in Table III.

Phylogenetic analysis clearly distinguished *H. pellis* from all *Henneguya* species sequenced to date, positioning it within a group of *Henneguya* known to infect ictalurid fishes, sister to *H. sutherlandi*, which exhibits a similar external presentation in channel catfish (Griffin et al., 2008). The 18S rDNA gene is used in molecular systematics for determining relationships within the Myxozoa because it is highly variable between very closely related species (Kent et al., 2001). Although pair-wise comparison of 18S rDNA sequence between these 2 species revealed only a 3% difference over nearly 2,000 base pairs, almost all of this variation is localized within the aforementioned diagnostic variable regions of the 18S rDNA locus and is in excess of the 1–2% that may be attributed to intraspecific variation (Whipps et al., 2003; Easy et al., 2005; Whipps and Diggles, 2006).

At present there is no established criterion that differentiates between intraspecific and interspecific variation in 18S rDNA sequence of the myxozoans. Nonetheless, based on available 18S rDNA sequence data, *H. sutherlandi* and *H. pellis* are not even the

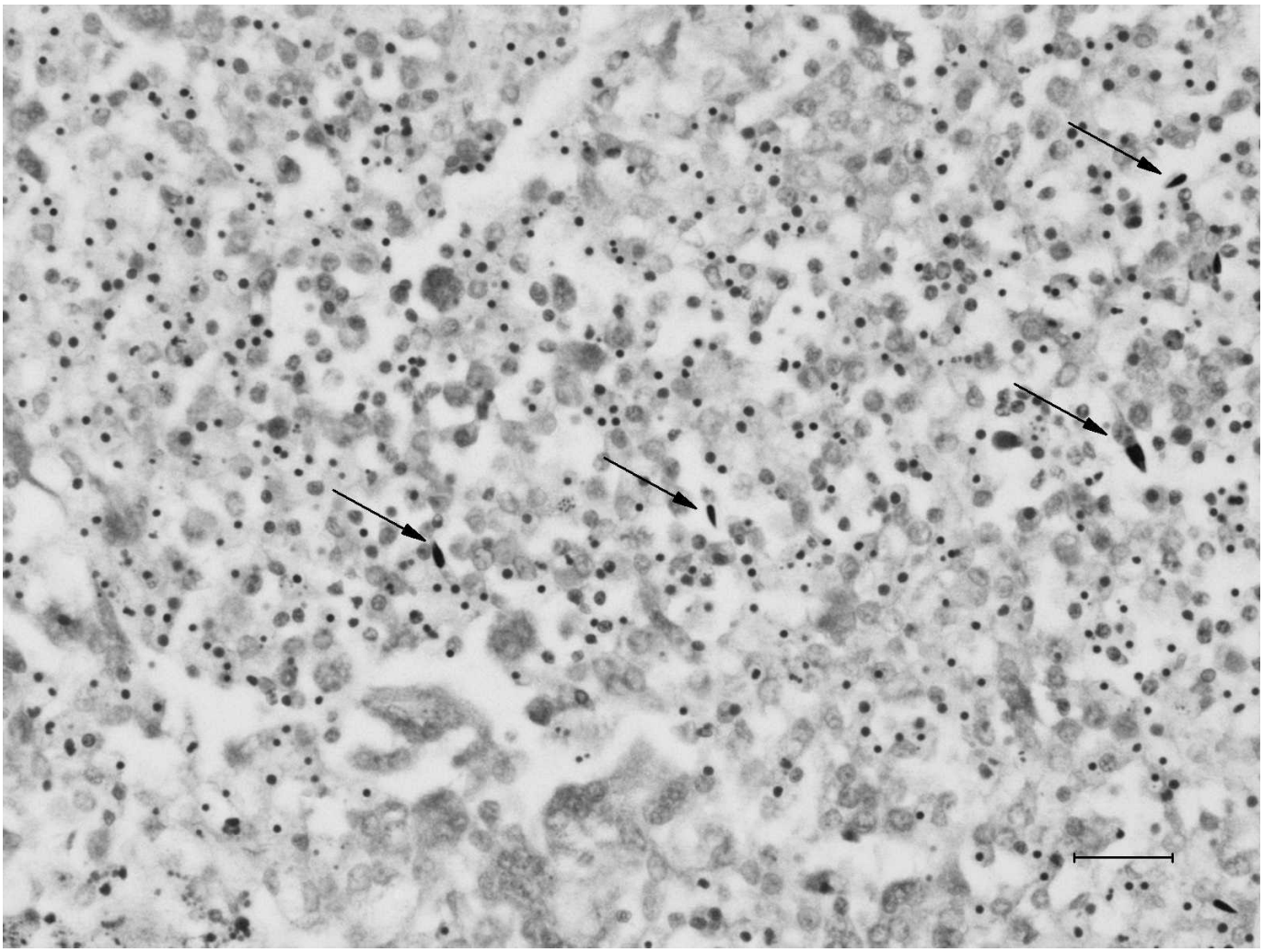

FIGURE 6. Higher magnification of the peritoneal pseudocyst. The arrows indicate the rare spores in the inflammatory milieu (Giemsa; scale bar ~25 μm).

TABLE II. Identity of *Henneguya pellis* small subunit ribosomal DNA (SSU rDNA) (Genbank accession number FJ468488) to other *Henneguya* species sequences. Sequence ambiguities were removed prior to calculation.

Species	Host family	DNA sequence identity to *H. pellis*	Genbank accession no.
H. sutherlandi	Ictaluridae	1,935/1,993 (97.1%)	EF191200
H. gurleyi	Ictaluridae	1,788/1,915 (93.4%)	DQ673465
H. adiposa	Ictaluridae	1,830/1,904 (91.6%)	EU492929
H. ictaluri	Ictaluridae	1,856/2,027 (91.6%)	AF195510
H. exilis	Ictaluridae	1,853/2,028 (91.4%)	AF021881
H. weishanensis	Percichthyidae	657/752 (87.4%)	AY165182
H. daoudi	Osphronemidae	842/977 (86.2%)	EU643625
H. doori	Percidae	1,761/2,060 (85.5%)	HDU37549
H. pagri	Gobiidae	1,780/2,101 (84.7%)	AB183748
H. akule	Carangidae	1,711/2,045 (83.7%)	EU016076
H. lesteri	Eleotridae	1,616/1,935 (83.5%)	AF306794
H. lateolabracis	Sillaginidae	1,712/2,043 (83.4%)	AB183747
H. shaharini	Lateolabracidae	860/1,035 (83.1%)	EU643630
H. rhinogobii	Sparidae	1,602/1,939 (82.6%)	AB447993
H. zschokkei	Salmonidae	1,511/1,937 (78.1%)	AF378344
H. salmonicola	Salmonidae	1,507/19,471 (77.7%)	AF031411
H. cutanea	Cyprinidae	1,239/1,615 (76.7%)	AY676460
H. neusslini	Salmonidae	1,092/1,426 (76.6%)	AY669810
H. doneci	Cyprinidae	690/909 (75.9%)	EU344899

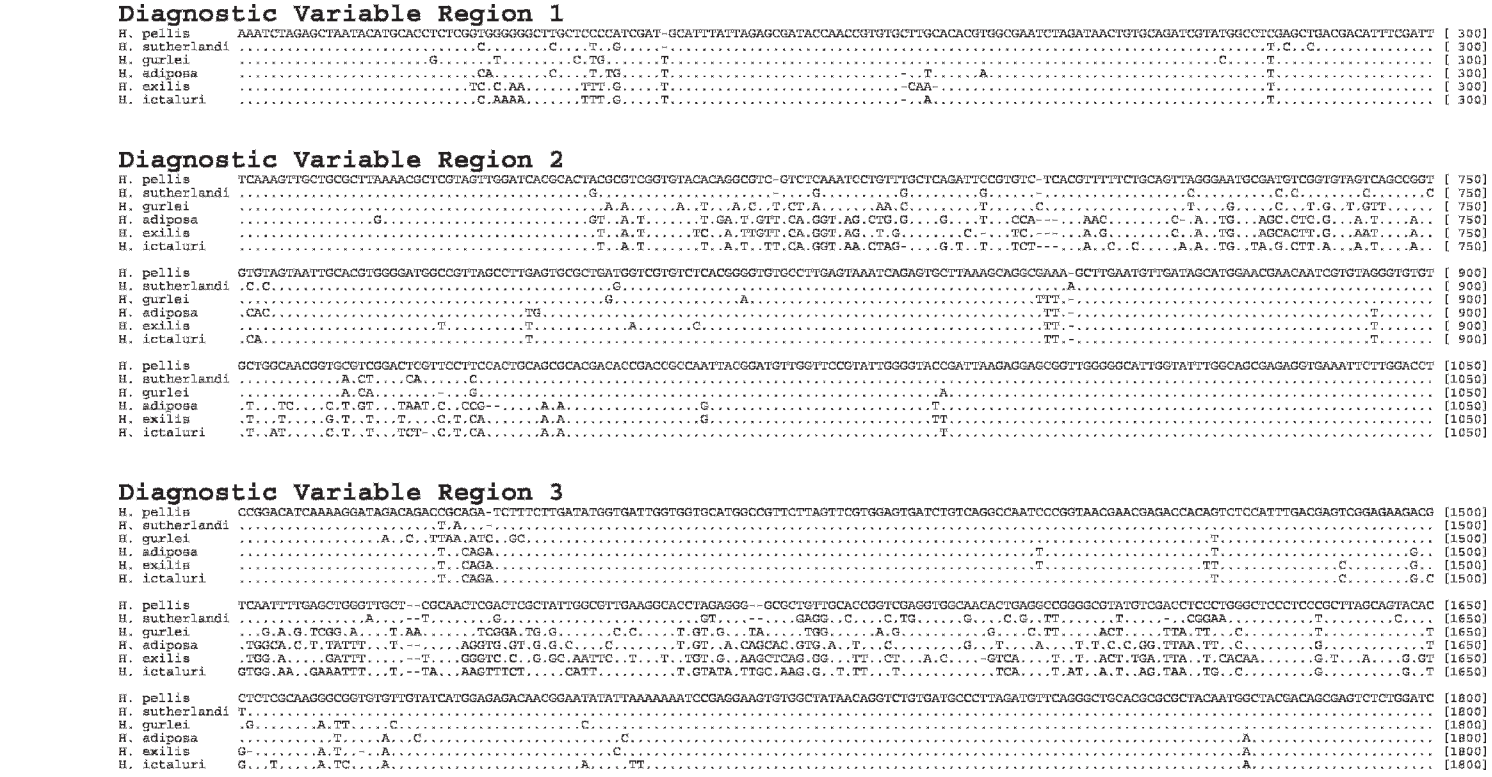

FIGURE 7. Alignment of 18S rDNA emphasizing the diagnostic variable regions. Nucleotide location is based on the 18S rDNA sequence of *Henneguya exilis* (AF021881).

most closely related members of the Myxobolidae. *Myxobolus elipsoides* isolated from the gills of roach (*Rutilus rutilus*) and *M. neurobius* from neural tissue of rainbow trout (*Oncorhynchus mykiss*) share greater than 97% sequence identity (Andree et al., 1999), as do *Myxobolus susanlimae* and *Myxobolus margitae* from the gills of bleak (*Alburnus alburnus*) and *Myxobolus feisti* from the gills of roach (Molnar et al., 2008). *Myxobolus procerus* and *Myxobolus intramusculi*, both isolated from the muscles of trout-perch (*Percopsis omiscomzycus*), differ by only 2.1% across 1,000 base pairs (Easy et al., 2005), and *Myxobolus pseudodispar*, *Myxobolus cyprinid*, and *Myxobolus musculi* share greater than 99% sequence similarity between them. Finally, *Myxobolus pendula* and *Myxobolus pellicides*, both isolated from creek chub (*Semotilus atromaculatus*), differ by only 8 bases across nearly 2,000 base pairs (99.6% similarity) (Kent et al., 2001). These data further support the original claim that *H. sutherlandi* and *H. pellis*, although similar in morphology and presentation, are in fact 2 separate species infecting different hosts (Griffin et al., 2008).

The morphological and genetic similarities between *H. pellis* and *H. sutherlandi*, coupled with the similar cutaneous presentation, suggest these 2 species arose from a single common ancestor. However, it is doubtful these *Henneguya* spp. would have arisen from a single common ancestor in the short time catfish aquaculture has been practiced. It is more likely they were both established species well before their introduction to the farm-raised catfish industry. Considering the nature of intensive aquaculture and the fact that blue and channel catfish are rarely commercially polycultured, it is possible the commercial catfish industry provided optimal conditions for the propagation and continued genetic separation of these 2 myxozoan species. Nevertheless, the data presented here do not provide enough evidence to definitively determine the mode of speciation.

Examination of the consensus trees indicates a clustering of the ictalurid-infecting *Henneguya* species on the basis of tissue predilection rather than host species. The 18S rDNA sequence of *H. sutherlandi*, isolated from the dermis of channel catfish, is most similar to *H. pellis* and *H. gurleyi* isolated from cutaneous tissues of blue catfish and brown bullhead, respectively, and not *H. ictaluri*, *H. exilis*, and *H. adiposa* isolated from channel catfish. This supports previous work claiming tissue predilection should be given as much consideration as host species when determining phylogenetic relationships (Eszterbauer, 2004). These data also suggest that host species should be considered when discriminating between morphologically similar organisms and, in some instances, may be a valid taxonomic characteristic.

On several occasions, researchers have discussed the pitfalls of using spore morphology and host record in discriminating between myxozoan species (Eszterbauer, 2002, 2004; Hogge et al., 2004, 2008; Iwanowicz et al., 2008). The asynchronous development and morphological variation in species of myxozoans that infect multiple hosts (Hallett et al., 2006) has led to several morphologically similar isolates from different hosts erroneously being described as the same species (Molnar et al., 2002). This has resulted in exaggerated host ranges for some myxozoans and called for the inclusion of host and tissue specificity in phylogenetic classifications, which often correlated better with 18S rDNA sequence data than spore morphology alone (Kent et al., 2001; Eszterbauer, 2004). Data presented here suggest, in the absence of DNA sequence data, that morphology, host species, and tissue predilection all play critically important

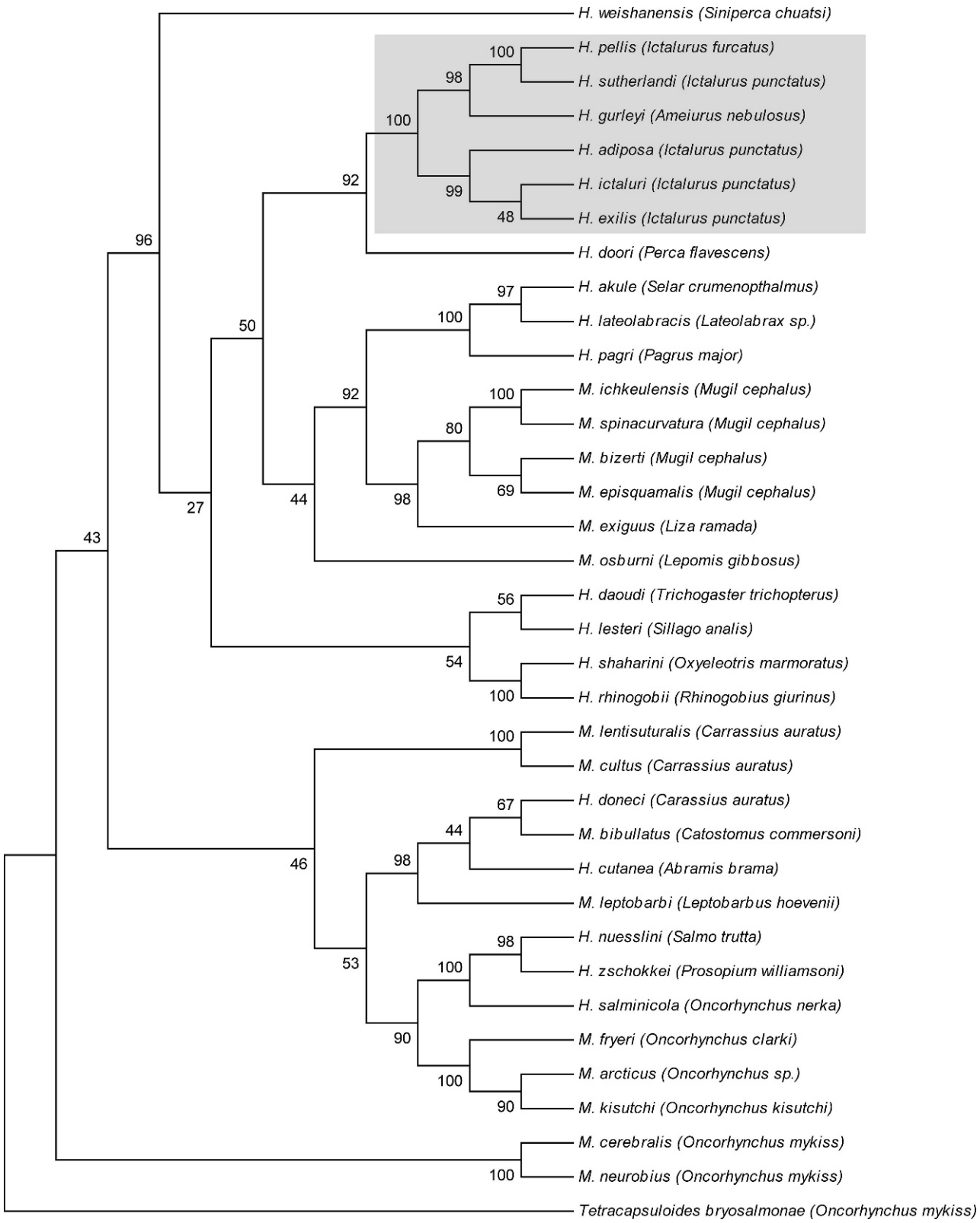

FIGURE 8. Phylogenetic tree generated by maximum parsimony of the 18S rDNA sequences of selected myxozoans (fish host), rooted at *Tetracapsuloides bryosalmonae*. Numbers at nodes indicate bootstrap confidence values (n = 100 replicates). Myxozoans isolated from ictalurid fishes are highlighted in gray.

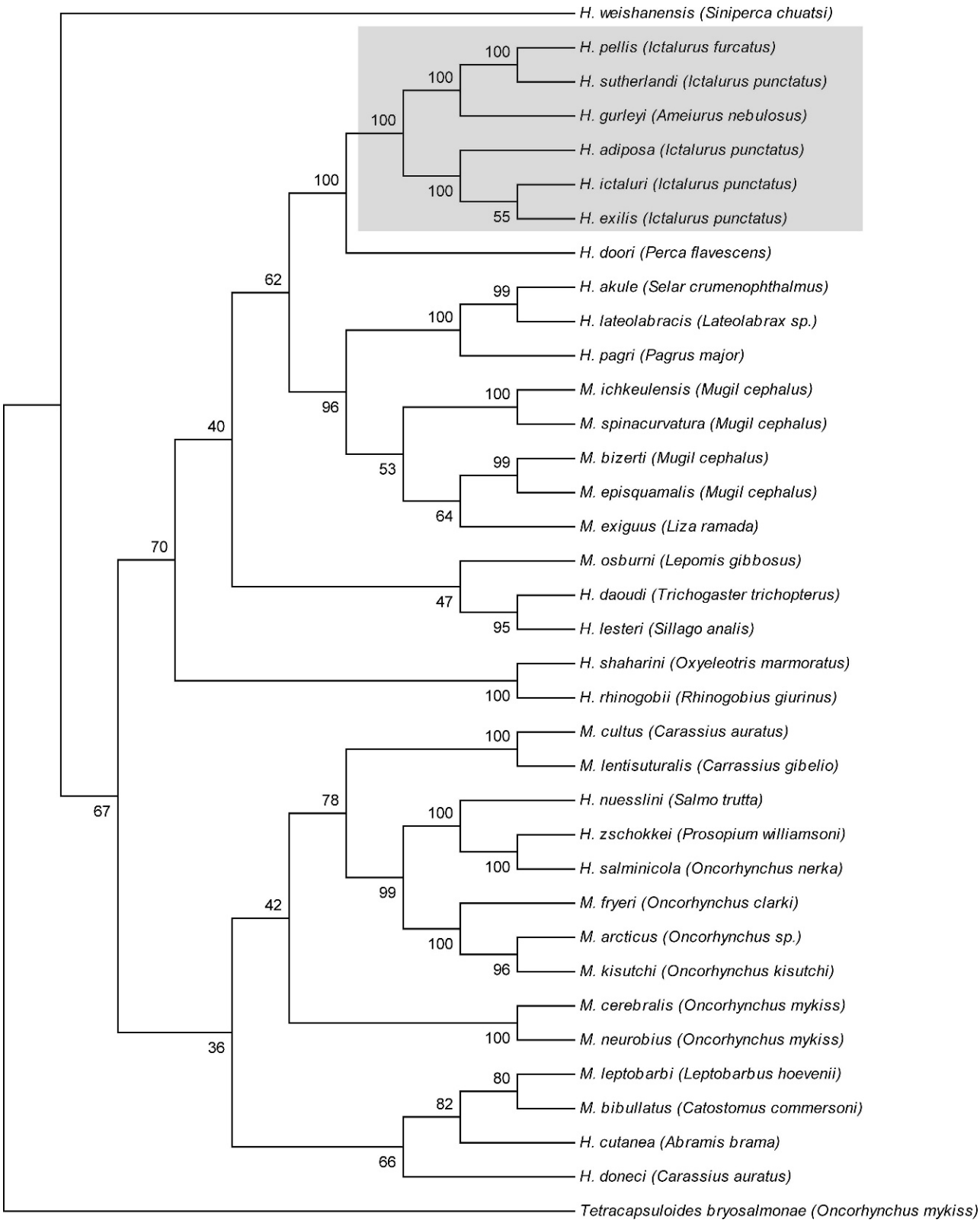

FIGURE 9. Phylogenetic tree generated by minimum evolution analysis of the 18S rDNA sequences of selected myxozoans (fish host), rooted at *Tetracapsuloides bryosalmonae*. Evolutionary distances were determined by maximum composite likelihood. Numbers at nodes indicate bootstrap confidence values (n = 1,000 replicates). Myxozoans isolated from ictalurid fishes are highlighted in gray.

TABLE III. Morphological characteristics of *Henneguya* species isolated from ictalurid fishes in the southeastern United States. LSB, length of spore body; WSB, width of spore body; LPC, length of polar capsule; WPC, width of polar capsule; CPL, caudal process length; TSL, total spore length; CD, cyst dimensions; N/A, data not available. All measurements are provided in microns (μm) except for the cyst dimensions (mm). Values in parentheses denote size range.

Species	LSB	WSB	LPC	WPC	CPL	TSL	CD	SI	Host	GenBank accession no.	Reference
H. pellis	14.8 (13.0–17.1)	4.7 (4.0–5.7)	7.2 (6.2–8.4)	1.7 (1.4–1.9)	77.7 (57.4–96.4)	92.5 (73.3–113.5)	3–5	Skin and body wall of the peritoneal cavity	*Ictalurus furcatus*	FJ468488	This paper
H. pellis	13.0 (11.0–14.5)	5.0 (4.5–5.2)	6.9 (5.5–8.5)	1.8 (1.5–2.0)	87.8 (66–112)	100.4 (79–124)	1–2	Skin	*Ictalurus furcatus*	N/A	Minchew, 1977
H. sutherlandi	15.4 (12.2–19.3)	5.5 (4.5–6.8)	6.1 (4.0–7.9)	1.7 (1.0–2.2)	50.5 (34.8–71.4)	60.3 (50.6–69.1)	1–2	Skin	*Ictalurus punctatus*	EF191200	Griffin et al., 2008
H. diversis	14.8 (13.5–16.5)	4.0 (3.2–5.0)	6.2 (6.0–7.5)	1.5 (1.0–2.0)	34.6 (25–47)	49.5 (40–62)	Up to 0.25 × 0.6	Base of barbels, pectoral fins and along isthmus, liver and kidney	*Ictalurus punctatus*	N/A	Minchew, 1977
H. postexilis	15 (13.5–17)	3.4 (3.5–4.0)	6.6 (5.9–7.2)	1.5 (1.0–2.0)	37.0 (28–49)	52 (42–62)	(0.012 × 0.012–0.08 × 0.07)	Gills	*Ictalurus punctatus*	N/A	Minchew, 1977
H. ictaluri	23.9 (20.8–26.1)	6.0 (4.5–6.4)	8.1 (7.6–9.6)	2.5 (2.0–3.2)	63 (48.1–80.2)	N/A	N/A	Gills	*Ictalurus punctatus*	AF195510	Pote et al., 2000
H. exilis	(18–20)	(4–5)	(8–9)	(1.0–1.5)	N/A	(60–70)	(0.5–2)	Gills	*Ictalurus punctatus, Ameiurus melas, Ameiurus nebulosus*	AF021881	Kudo, 1929; Lin et al., 1999
H. gurleyi	18.2 (15.7–20.3)	5.4 (3.8–6.1)	5.9 (4.8–7.1)	1.2 (1.0–1.5)	41.1 (34.0–49.9)	60.9 (48.7–68.5)	Up to 1.8	Dorsal, pectoral, and anal fins	*Ameiurus nebulosus*	DQ673465	Kudo, 1920; Iwanowicz et al., 2008
H. longicauda	16.2 (14–17.5)	4.0 (3.4–4.5)	7.7 (7.0–8.5)	1.8 (1.5–2.0)	90.5 (75–110)	108.3 (91–127)	(0.13 × 0.37–0.11×0.12)	Gills	*Ictalurus punctatus*	N/A	Minchew, 1977
H. ameiurensis	23.3	4.1	5.4	1.6	(15–41.5)	N/A	(0.19 × 0.34–0.76 × 1.2)	Barbels	*Ameiurus nebulosus*	N/A	Nigrelli and Smith, 1940
H. adiposa	17.1 (14.7–20.5)	4.1 (3.4–4.6)	7.2 (5.8–8.3)	1.3 (0.9–1.9)	38.0 (23.2–48.8)	55.6 (40.7–65.8)		Adipose fin	*Ictalurus punctatus*	EU492929	Griffin et al., 2009
H. adiposa	16.3 (12–19)	4.0 (3.5–5.0)	7.7 (6.2–9.0)	1.5 (1.0–2.0)	44.8 (28–59)	61.0 (45–75)	(0.12 × 0.29–0.5 × 0.15)	Adipose fin	*Ictalurus punctatus*	N/A	Minchew, 1977

roles in phylogenetic classifications. However, no characteristic appears favorable over the others, since phylogenetic classifications based on a single characteristic, such as the presence of elongate caudal processes, could result in several different groupings, most of which do not correlate with molecular sequence data (Kent et al., 2001). As a consequence, molecular techniques are often the only means for discriminating between morphologically indistinguishable species from various hosts and tissues (Salim and Desser, 2000; Molnar et al., 2002; Hogge et al., 2004; Cone et al., 2005; Easy et al., 2005; Whipps and Diggles, 2006; Griffin et al., 2008; Hogge et al., 2008; Iwanowicz et al., 2008; Ferguson et al., 2008; Molnar et al., 2008). As such, it has been suggested on several occasions that molecular sequence data should be included when new species are described (Kent et al., 2001; Lom and Dyková, 2006; Whipps and Diggles, 2006).

The data presented here also indicate these ictalurid-infecting *Henneguya* species are more closely related to species of *Myxobolus* than *Henneguya* species isolated from other host families. This supports previous claims that *Myxobolus* and *Henneguya* have a monophyletic origin (Kent et al., 2001; Fiala, 2006; Lom and Dyková, 2006). The considerable geographic separation and variability in preferred habitats of these different host families, coupled with the significant genetic separation between the ictalurid-infecting *Henneguya* species and those infecting fishes of other geographic locales, suggests that paired caudal processes have developed during separate evolutionary events within the Myxobolidae. However, at this time, the selective pressures that led to the development or loss of these elongated caudal processes remain unclear.

For most of the ictalurid-infecting *Henneguya* species, the fish host demonstrates limited to no reaction to the mature myxospores, and most damage is incurred during the initial attachment, penetration, and development of the actinospore stage of the organism (Pote et al., 2000; Wise et al., 2004). In the limited number of fish examined, *H. pellis* appears to produce limited, localized pathology in the fish host; however, the impacts of heavy infections on the health and performance of fry and fingerlings or the predisposition to secondary infections following initial penetration of the actinospore stage or rupture of the dermal pseudocysts is unknown. Similar to *H. sutherlandi* in channel catfish, localization of the parasite in the dermis and peritoneal wall suggests they would be removed during processing; however, the effects of heavy infections on the health and marketability of food-size fish remain unclear (Griffin et al., 2008).

This research supplements the original description of *H. pellis* (Minchew, 1977) with photomicrographs, line drawings, histopathology, and molecular sequence data, identifying *H. pellis* as a distinct species from *H. sutherlandi*. This is also the first mention of plasmodial development on the body wall of the peritoneal cavity, a presentation that was not documented in the original description (Minchew, 1977). The slight differences in measurements between our observations and those of the previous author can most likely be attributed to the asynchronous development of the parasite or artifacts of fixation, not to mention technological advancements in optics, digital imagery, and computer assisted measurements developed since 1977. The 18S rDNA sequence of *H. pellis* has been submitted to Genbank (accession number FJ468488), and infected tissues (preserved in 95% ethanol) will be deposited in the parasitology collection of the Queensland Museum, Brisbane, Australia.

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